#20 Response

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Murphy et al.

Application Serial No. 09/554,431

Filed: May 15, 2002

Group Art Unit: 1655

Examiner: B. Sisson

For: IMPROVEMENTS IN OR RELATING TO DETECTION OF MOLECULES IN SAMPLES

July 29, 2002

PRELIMINARY AMENDMENT

Hon. Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to further prosecution on the merits pursuant to the Request for Continued Examination filed concurrently herewith, please note the following remarks.

REMARKS

The Examiner states that it would be difficult to distinguish between exogenous administered and endogenous polypeptide "when the exogenously administered polypeptide fluoresces at the same wavelength as does the endogenous polypeptide."

The Examiner is correct that both endogenous and exogenous polypeptides may fluoresce at the same wavelength. However, due to the difference in the number of fluorophores between the endogenous and exogenous polypeptides (as required by the invention), particularly tryptophans, it is fairly simple to differentiate between them.

Proteins contain three aromatic amino acid residues (tryptophan, tyrosine, phenylalanine) which contribute to the polypeptides intrinsic fluorescence.

The fluorescence of a folded protein is a mixture of the fluorescence from the individual aromatic residues. As described in the patent application, due to its high

absorption and fluorescence quantum yield (emitted photons/absorbed photons), trytophan is much more fluorescent than tyrosine, while phenylalanine is only weakly fluorescent (see Table I).

Table I: Summary of the fluorescence characteristics of the three aromatic residues.

	Absorption		*Fluorescence*	
Amino Acid	Wavelength	Absorptivity	Wavelength	Quantum
Tryptophan	280	5,600	348	0.20
Tyrosine	274	1,400	303	0.14
Phenylalanine	257	200	282	0.04

Consequently, when a polypeptide is excited, which is generally at 280 nm or at longer wavelengths, usually at 295 nm, most of the emissions are due to excitation of tryptophan residues, such that the fluorescence spectrum of a polypeptide containing the three residues usually resembles that of tryptophan.

It will be understood, therefore, by those skilled in the art that, due to the very strong fluorescent properties of tryptophan, the addition, or absence, of just a single tryptophan residue will have a marked effect on the fluorescent spectra around the maximum emission for tryptophan, *i.e.*, 345 nm.

As another example, the 'tagged' exogenous human growth hormone has two additional tryptophans compared to the 'untagged' endogenous human growth hormone. It has been confirmed that the 'tagged' growth hormone has a much stronger fluorescence at 345 nm than the 'untagged' polypeptide, as expected.

It is further clear that the combined concentration of 'tagged' exogenous and 'untagged' endogenous polypeptide can be readily quantified using prior arts techniques. As the fluorescence of a polypeptide excited at around 290 nm effectively correlates to the molar concentration of tryptophans in the molecule, given the experimentally determined total concentration of the polypeptide in a sample, an expected fluorescence (345 nm) can be calculated assuming 100% of the endogenous 'untagged' polypeptide. Any deviations from the calculated

fluorescence are, therefore, due to the exogenous 'tagged' polypeptide, and the concentration of the 'tagged' exogenous polypeptide can readily back-calculated.

Moreover, the examples provided in the specification are based on existing molecules. For the 'untagged' endogenous polypeptides, methods of manufacture, formulation and clinical protocols exist, some probably patented by their innovators. To fluorescently 'tag' a known polypeptide without changing its physiochemical properties, existing methods of manufacture, formulation and administration should essentially be identical to the manufacture of the 'untagged' polypeptide.

Therefore, contrary to assertions in the Advisory Action and final rejection, it is feasible and practical, without undue experimentation, to differentiate between endogenous and exogenous polypeptide, as described in the specification.

Regarding the "written description", wherein it is stated that "the specification does not reasonably suggest that applicant was in possession of a method whereby not only were the exogenously administered compounds to be fluorescent, but that they had also undergone centrifugation, affinity chromatography, immuno-affinity chromatography, denaturation and/or heat treatment", Applicants strongly disagree. Not only were all of these methods available well before the time of filing of the application, but the disclosure clearly conveys that Applicants were in possession of this subject matter, not only by the original claim language but also by the presentation of prophetic examples. Since the techniques were well known, there is no obligation for the specification to provide a detailed blueprint on the specifics.

Further, for any polypeptide approved for clinical use, by default, it must have been through an extremely extensive characterization program *in vitro* as well as *in vivo*. As such, methods for its enrichment, such as affinity chromatography, Hplc and Fpl will exist.

In any case, it is respectfully submitted that the Examiner is missing the focus of the claims. What is claimed is a method of differential detection, not a method of making 'tagged' polypeptides. The prior art adequately describes that the fluorescence of proteins is altered by the addition or subtraction of tryptophans and that the method of manufacture, formulation and therapeutic use of existing polypeptides, particularly growth hormone, are extensively described in the prior art.

Since the claims are free of the prior art and in compliance with all other statutory requirements, passage of the application to issue is earnestly solicited.

Respectfully submitted,

PILLSBURY WINTHROP LLP

By: Richard A. Steinberg

Richard A. Steinberg
Registration No. 26,588
Direct No. (703) 905-2039

1600 Tysons Boulevard
McLean, Virginia 22102
(703) 905-2000 Telephone
(703) 905-2500 Facsimile

Attorney Reference: 041301/0268066